Final Minutes of the National Toxicology Program (NTP) Advisory Committee on Alternative Toxicological Methods (ACATM) Meeting

March 7 - 8, 2000

National Institute of Environmental Health Sciences (NIEHS)
Building 101
Research Triangle Park, NC

The National Toxicology Program (NTP) Advisory Committee on Alternative Toxicological Methods (ACATM) met on March 7 & 8, 2000, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina.

The following ACATM members were in attendance:
Katherine A. Stitzel, D.V.M. (chair), Procter & Gamble Company, Cincinnati, OH
Paul T. Bailey, Ph.D., Exxon Mobil Biomedical Sciences, Inc., Annandale, NJ
Rodger Curren, Ph.D. (*ad hoc* member), Institute of *In Vitro* Sciences, Inc., Gaithersburg, MD
Michael S. Denison, Ph.D., University of California—Davis, Davis, CA
Alan M. Goldberg, Ph.D., Johns Hopkins University, Baltimore, MD
Sidney Green, Ph.D., Howard University College of Medicine, Washington, DC
Wallace Hayes, Ph.D., Gillette Company, Boston, MA
Susan Hurt, Ph.D., Rohm and Haas Company, Spring House, PA
Roger McClellan, D.V.M., retired from Chemical Industry Institute of Toxicology, Research Triangle Park, NC
Charles Montgomery, D.V.M., Baylor College of Medicine, Houston, TX
Kenneth Ramos, Ph.D. (*ad hoc* member), Texas A&M University, College Station, TX
Andrew Rowan, Ph.D., Humane Society of the United States, Gaithersburg, MD
Peter Theran, D.V.M., Massachusetts Society for the Prevention of Cruelty to Animals, Boston, MA

The following ACATM member was not in attendance:
Elaine Faustman, Ph.D., University of Washington, Seattle, WA

Other Meeting Attendees:

Amersham Pharmacia Biotech, Ltd.
Dr. Liz Kasber

Doris Day Animal League
Ms. Sara Amundson

Duke University
Dr. Simon Lin

Dynamac
Dr. Steven Brecker

Environmental Protection Agency
Dr. Carl Blackman
Dr. Ethel Derr-Yellin
Dr. Tony DeAngelo
Dr. David DeMarini
Dr. Joe Elder
Dr. Zumi Feng
Dr. Kirk Kitchin
Dr. James Rabinowitz
Dr. Ann Richard

Dr. Defa Tian
Dr. Juan Zhang

Food and Drug Administration
Dr. Leonard Schechtman

GenTec International
Dr. Marilyn Stapleton

Glaxo Wellcome, Inc.
Dr. Scott Sundseth

Integrated Laboratory Systems, Inc.
Dr. Elizabeth Brown
Dr. Finis Cavender
Dr. Tom Goldsworthy
Dr. Barry Margolin
Dr. Ray Tice

Janssen Pharmaceutical Research Foundation
Dr. Bruce Ruoff

National Institute of Environmental Health Sciences
Mr. Christopher Borchers
Dr. John Bucher
Mr. Pierre Bushel
Ms. Monica Divers
Ms. Loretta Frye
Dr. Jerry Heindel
Dr. Jie Liu
Dr. Tong Lu
Dr. George Lucier
Dr. Richard Paules
Dr. Scott Masten
Ms. Debbie McCarley
Dr. Alex Merrick
Dr. Sheila Newton
Dr. Jud Spalding
Dr. William Stokes
Dr. Ken Tomer
Dr. Nigel Walker
Dr. Mary Wolfe

People for the Ethical Treatment of Animals (PETA)
Ms. Mary Beth Sweetland

Xenobiotic Detection Systems, Inc.
Dr. George Clark

Tuesday, March 7, 2000

I. Welcome and Introduction

Dr. Katherine Stitzel, Chair of ACATM, called the meeting to order at 1:00 p.m. and asked everyone to state his/her name and affiliation for the record.

Dr. Wolfe stated that the meeting was being taped and that summary minutes would be prepared. She then read the Statement on Conflict of Interest: "The members of the NTP Advisory Committee on Alternative Toxicological Methods serve as individual scientists and not as representatives of any organization. Each member is to exercise judgment prior to any meeting as to whether a potential conflict of interest might exist, relative to one or more of the topics being discussed due to his or her occupation affiliation, professional activity, or financial interest. Should there be a potential conflict of interest, the member is to decline service as a principal reviewer and abstain from any vote. The member is not precluded from otherwise participating in the discussion."

Dr. Lucier thanked the two retiring board members for their service to the Committee, Drs. Hurt and Montgomery. He expressed the NTP's and the Institute's appreciation for their hard work, noting that they served as charter members of this new board. He added that extraordinary progress has been made in meeting the objectives of developing ways to validate new methods in toxicology, and recognized the Committee for their contribution to this effort. He then presented certificates of appreciation and letters to Drs. Hurt and Montgomery. He acknowledged two *ad hoc* members in attendance, Dr. Ramos and Dr. Curren and thanked them for attending.

Dr. Lucier stated that the Committee previously asked the NTP to start thinking about some of the new evolving technologies that might eventually lead to alternative tests in toxicology. This includes the generation and use of genomic information, much of it emerging from the human genome project. He stated that there is a need to begin to consider how this would be used in toxicology and how it might impact new tests. For instance, this information will certainly be used in priority setting. He also noted as more information is generated from systems such as gene microarrays, there is no doubt that eventually information will be used for hazard identification. These methods are clearly in the early stages of development, and this is the time to start thinking about validation issues, and how various agencies might use this information in their regulatory decision-making process and in setting public health policy. Dr. Lucier noted that concept of mechanism-based toxicology has been a centerpiece this decade for the NTP. The NTP had a conference in 1995 to look at mechanism-based toxicology and it's role in understanding how various agents cause toxicity. The workshop addressed how such information might be used to reduce uncertainties in risk assessment, particularly those related to dose response and hazard identification. He added that new models will emerge from the genome project that will be discussed by the presenters that follow. He concluded by adding that the NTP values the Committee's comments regarding what the NTP needs to be thinking about in terms of experimental design and other issues to consider during this early phase of development.

Dr. Lucier then introduced Dr. Rick Paules, NIEHS, who was hired recently by the Environmental Toxicology Program in a toxicogenomics position to coordinate information emerging from the genome project and related technologies, and to advise on how to incorporate that kind of information into NTP priority setting. Dr. Paules organized the session in order to provide information about NTP's initiatives in toxicogenomics and microarray technology.

Dr. Stokes added that there are enormous opportunities for applying this genomic technology to develop test methodologies that are more predictive, and that it was important to begin thinking about how to validate such methods. He noted that, based on the Center's experience, the established validation and acceptance criteria needs to be considered early and not after extensive studies have been completed.

I. Application of Emerging Technologies to Toxicology Testing: Gene Expression Assays

A. Introduction and Overview of the Gene Expression Assays and Microarray Technology; Application of Gene Expression Patterns to *In Vitro* and *In Vivo* Toxicological Assessments - Dr. Richard Paules, NIEHS, Division of Intramural Research (DIR), Environmental Toxicology Program (ETP), Laboratory of Environmental Carcinogenesis/Mutagenesis (LECM), Growth Control and Cancer (GCC), presented information related to toxicogenomics at NIEHS. He stated that the premise of the toxicogenomic work was to understand the impact of the environment on the function of genes, which would allow a better understanding of the impact of the environment on human health. He defined toxicogenomics as the identification of potential toxicants, and their putative mechanism of action, through the use of genomic resources. The key challenges of environmental health studies, as explained by Dr. Paules, deal with determining how the impact of exposures to environmental insults can be accurately evaluated in the context of human health. Through the toxicogenetics program, NIEHS has taken an integrated approach to gene expression. First, areas of expertise currently available at NIEHS that can be used now or in the future to further technological advances in the field are identified. Second, research goals are developed that provide a focus for these areas as a unified effort. These steps allow for the creation of an integrated, collaborative approach to the research goals, which will benefit from the synergy of the collective effort.

Dr. Paules stated the following as the functions of the NIEHS Microarray Center:

- Design a complimentary deoxyribonucleic acid (cDNA) microarray facility to support environmental health research needs;
- Create unique gene arrays and subarrays for production of cDNA microarray chips;
- Develop data management and analysis systems; and
- Determine the impact of environmental challenges on patterns of gene expression.

Dr. Paules provided an overview of analyses using cDNA microarrays, including how the data are collected, the software used to collect and analyze the data, and an example of the resulting image analysis. He then discussed the cDNA microarrays and chips that are currently used at NIEHS. These include the three human gene chips (i.e., the Human Toxchip, the Human Discovery Chip, and the Human Senescence Gene Chip), as well as chips using yeast, mouse, rat, and *Xenopus* genes. He also explained the role of bioinformatics within the Microarray Center. Bioinformatics serves as the tool for information management and organization. It is used to develop clone and array databases, to manage data and perform statistical analysis, and to interface with the Center web page and the web server. Microarray studies currently in progress at NIEHS include those testing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), estrogen, hydrogen peroxide, γ-irradiation, arsenic, paraquat, and Phenobarbital in human cell cultures. MMS and γ-irradiation are also being tested in yeast cells, and TCDD, peroxisome proliferators, and phenobarbitol are being tested in cultured rat liver cells.

Discussion:

Dr. McClellan asked if the Institute has a strategic plan for evaluating gene expression patterns for "known toxicants." Of particular interest would be the evaluation of materials with known toxicity profiles from human experience, as well as profiles from laboratory animal data. He

asked Dr. Paules to comment on that strategy. Dr. Paules responded that discussions on that topic are being held within the NTP and the NIEHS Microarray Center, and that he is really excited about bringing outside participation into this effort. The NIEHS Microarray Center would like to be the repository for high quality toxicological studies, both from our Institute, academia, the private sector, and industrial partners. They are in the process of conducting a number of studies with pharmacological companies and have some industrial partners. He sees the field moving slowly at first as we get a feel for what doses and what compounds are the most informative. There will be a huge robust dataset that will be built and the data will tell what is the best learning set. For example, it is not known whether it is most informative to look at one, three, seven, fourteen or twenty-one days, but studies are being conducted to evaluate that. In terms of classic compounds, there is a lot of discussion as to what are the best learning sets. There will be a lot of information that will come as a collective effort from the scientific community and he feels that the actual data will reveal what is most informative.

Dr. McClellan commented that he agreed to a certain extent, but thinks that certainly NIEHS/NTP has a wealth of experience in the area of validation of new test systems. He stated it was important to draw on all of the experience gained from genetic toxicity and much could be learned from the approaches for validation of some of those activities. He emphasized the a need for a strategy to guide what is being done, and underscored the importance of trying to create a strategy, not just letting it "evolve." He asked how the Microarray Center was addressing the issue of potency, dose-response slope, and the extent to which we could make a comparison between five compounds and selecting the dose. Dr. Paules responded that dose range finding types of experiments are being conducted. These are high dose exposures and in these gene expression studies of acute response, maximal numbers of genes change in response to high dose. The question is whether there is specificity, or only a generalized toxic response. This is something that can be sorted out by looking across classes and by dropping doses.

Dr. McClellan stated a concern about the use of the term "risk" as we know that risk takes into account both the potency of the material and the exposure. Microarrays may be very sensitive tools to identify hazard. However, these are hazard oriented, and how we ultimately are going to be able to move them into the world of risk assessment is a challenge. Part of the argument for a clearly articulated strategy applies to Dr. Paules' slide which notes: "identify research goals that can only be accomplished through a unified effort." He stated that a particularly important role for NIEHS to provide is leadership to do things that in most cases won't be done in the academic community where people don't have the kind of resources that can be marshaled at NIEHS. He expects that most of their effort will focus on hazard. NIEHS can build a bridge into the world of risk and he urged the Institute to start thinking in terms of creating a strategy, not just counting on bringing together that information for the rest of the community.

B. Application of Gene Expression Technology to Drug Development - Dr. Scott Sundseth of Glaxo Wellcome, Inc. provided an industry viewpoint on the application of gene expression technology for drug development. He explained that gene expression technology is used by the pharmaceutical industry in: target, surrogate, and pathway drug discovery; target validation and disease association; compound screening and optimization; evaluating mechanisms of action; toxicogenomics; and pharmacogenomics. Examples of technologies currently being used at Glaxo Wellcome include: polymerase chain reaction (PCR); nylon, glass slide, and Affymetrix chips/arrays; CuraGen; and proteomics. Dr. Sundseth then provided an example of data, pattern analysis, compound characterization, and pathway discovery for compound regulation of fatty acid oxidation. He concluded by mentioning that gene-specific diagnoses have considerable potential for use in the drug development process. He felt that this technology would have a strong beneficial impact on drug research.

C. Real Time and Quantitative (RTAQ)-PCR - Dr. Nigel Walker, NIEHS, DIR, ETP, Laboratory of Computational Biology and Risk Analysis (LCBRA), provided an overview of

RTAQ-PCR. He explained that RTAQ-PCR provides fluorescence detection of PCR in real-time without the use of gels, discussed the utility of RTAQ-PCR, and its uses in toxicology studies. Primarily, RTAQ-PCR is used for hazard identification, quantitative dose-response assessment, and determination of concordance between species. He provided a schematic of the indirect and direct methods of fluorescence detection, as well as information pertaining to software-based analysis of the data. He then discussed pre-developed assays that are available at NIEHS and commercially. These include peroxisome proliferator-inducible receptor genes, PAH-inducible AHR receptor genes, and DNA damage-inducible genes, all of which have been developed at NIEHS. Those developed commercially include cytokines, cytokine receptor, and cell surface marker assays; chemokine and chemokine receptor assays; human cell and growth regulator assays; human apoptosis markers; and endogenous controls. Further information on the commercial assays is available at the following web site: **http://www.appliedbiosystems.com/ab/about/pcr/sds/ptar/targets.html.**

Dr. Walker explained the types of quantitation, which includes absolute quantitation that requires a standard curve and is used for multiple samples where one or two genes per 96-well plate are quantitated, and relative quantitation. Relative quantitation assesses the difference between control and unknown samples. This method either compares the expression of one gene in multiple samples or compares multiple genes in a few samples. Dr. Walker presented some work currently in progress, which includes dioxin and phthalate studies. Lastly, he provided a list of informational sources for the Taqman/SYBR green assays and Molecular Beacons. In addition to the web site previously mentioned, the following websites also provide information on the topic: **http://wwwstratagene.com/q_pcr/mb_intro.htm** and **http://lifetech.com/world_whatsnew/us_1_55.html**.

Discussion:

Dr. McClellan asked Dr. Walker to elaborate on the quantitative dose responses assessment: "low dose extrapolation dose response behavior," stating that he was interested in hearing his position about the strategy that will enhance the understanding of low-dose extrapolation for populations of animals.

Dr. Walker responded that these kinds of data can be used to provide dose-response behavior for certain endpoints that may be mechanistically related to the particular toxic endpoint. In terms of moving to human dose-response studies, that is much more complicated in that there is a lot more variation. The quantitative aspects of an assay are needed to reduce some of the inherent variability between individuals. In terms of going to low-dose extrapolation beyond the range of the experimental data, our experiences with the likes of dioxin are that you can't. The further you go beyond the range of that data even for something quantitative, you start getting very variable estimates, but still the quantitative aspects of this technology are that you can only get really low-dose estimates of effective 1% doses if you are using something quantitative like this. If you use other technologies, you don't have the sensitivity and therefore the effective doses that you do derive from analyses are a lot higher and they may be misleadingly high. You know that you get an effect that could occur at 1% effect level at lower doses, but using another technology, may require a much higher dose to produce a measurable effect.

Dr. McClellan responded that Dr. Walker's answer reflects that we really don't have a very good idea of how we are going to do low-dose extrapolation, and so we all continue to say that these technologies are going to do this. He thought it might be useful for NIEHS to consider a workshop to bring people together to move beyond the stage of discussing what the technology is going to do, and to generate ideas as to how we might apply it and then validate it. Certainly NTP has a wealth of data in terms of observations in animals. One should now start to think about the new models and how would we take this kind of technology and move downwards just as you have in some other areas where you have made predictions for humans and forget about

species to species extrapolation. He expressed that we are at the edge of a technology phase where we are overwhelmed with the opportunities of technology and where we are just starting to get some data in hand. He emphasized that it is time to start talking about what kind of strategy we will really need to use to address some of these critical issues.

Dr. Lucier responded that a workshop might be a good idea. He stated that there is often a lot of information on chemicals at the molecular levels, where early changes occur at low doses. When a substance interacts with a receptor and the early transcriptional events follow. However, there is often little information about the toxicities of the particular agent at higher doses. What we don't have is that connection between the two, and that is really the knowledge gap that creates difficulty in validating a pattern of gene expression changes for use in quantitative risk assessment. That knowledge gap will never be totally filled, but at least we can begin to obtain information about what are the key genes that are responsible, and patterns of genes that have to be activated to tell the cell to divide or to go into differentiation, rather than just looking at the response in isolation.

Dr. Lucier added that the advantage to this type of approach is that one can often get an idea of which group of genes needs activation, which are the key players, and then begin making connections with those particular genes rather than a single gene, which oftentimes communicates very little about quantitative risk assessment.

Dr. Montgomery agreed on the existence of a big gap and bridging that gap is no easy task, but we are presently much closer than in the past due to better use of molecular tools and the use of genetically engineered rodents to clarify several in-between steps by specifically knocking out an enzyme or manipulating a gene. He stated there still is a need for the whole body system. Genetically engineered models are becoming more sophisticated. Some of the larger pharmacutical companies are cross-breeding transgenics to obtain more detailed information. Bi-transgenics, tri-transgenics, etc. open a completely new realm of information attributed to knowledge gained from phenotyping of two separate animals; upon breeding of these two animals together, the relationship is not necessarily synergistic and the mechanistic part of the disease has to be somewhat redefined. Dr. Montgomery encouraged us not to overlook a complete biological system as a part of this process. Further, he stated that for these tools being described today, many of which he has used in his lab, the reproducibility of these tools was terrible in the early stages of the equipment development.

D. Proteomics – Drs. Alex Merrick, NIEHS, DIR, Environmental Disease and Medicine Program (EDMP), Laboratory of Molecular Carcinogenesis (LMC) and Kenneth Tomer, NIEHS, DIR, Environmental Biology Program (EBP), Laboratory of Structural Biology (LSB), presented information pertaining to Proteomics in the NIEHS Gene Expression Center. Dr. Merrick began by describing the "What, Why, and How" of proteomics. He discussed the routes to protein identification, variations in protein structure that affect separation and analysis, the separation of proteins using 2D PAGE, and the applications and analysis of Proteomic studies. Dr. Tomer discussed the micro-characterization of proteins and presented information related to the identification of SDS-PAGE and 2D PAGE separated proteins by mass spectrometry (MS) using MS Peptide Map Fingerprinting/MS Sequence Tags to query genomic and protein databases. Dr. Tomer provided an example of matrix-assisted laser desorption/ionization (MALDI)-MS following in-gel digestion of SDS-PAGE separated proteins separated from liver microsomes obtained from rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). He reviewed the results of a peptide mapping-based database search showing that dioxin-induced cytochrome P450 enzymes could be identified by MS. He concluded by stating his and Dr. Merrick's goal of advancing the field of Proteomics within the NIEHS Gene Expression Center and to make this research capability available to DIR scientists.

E. Bioinformatics - Mr. Pierre Bushel, NIEHS, DIR, EDMP, LMC discussed bioinformatics in DNA microarray data management and analysis. He began with an overview of bioinformatics, its dependencies, and use for cDNA Microarray Technology.

He explained that bioinformatics comprises four primary areas:

I. The implementation of professional database engines, objects, and relationships to manage large volumes of data;

- The design and development of robust computer applications and software tools to process and analyze complicated data;
- The reliance on intricate biological systems to model network infrastructures for knowledge-based enterprise systems; and
- Utilization of integrated biological resources and advanced information technologies to discover heuristic knowledge about disease states and multi-cellular responses.

Mr. Bushel explained that the success of bioinformatics relies on expertise from multiple disciplines (including biology, statistics, mathematics, and computer science), collaboration with the scientific community, and appropriate resources.

Mr. Bushel presented a schematic representation of the NIEHS Microarray Center (NMC) system for image and data processing management, detailing the process of network management of pre- and post-processed information from the clients, and explaining the system for fault tolerance and data protection. He discussed the bioinformatics analysis process, which includes data evaluation, data preprocessing and filtering, data archiving and storage, data mining, and cluster analysis. He briefly discussed the software used (i.e., the ARRAYSUITE software) and how the software provides statistical analysis of the data. Mr. Bushel illustrated the concept of data mining and analysis clustering, providing examples of a gene expression profile and correlation, and scatter and cluster analyses. He provided a schematic of the NMC laboratory information management systems and examples of the MicroArray Project System (MAPS) database and the National Human Genome Research Institute (NHGRI) ArrayDB 2.0 database, which will be implemented at NIEHS.

Dr. Rodger Curren asked for clarification regarding the types of gatekeeping activities stating that it becomes extremely complicated to identify and compare expression along with everything else; however, at some level, one must be certain that the protocol was followed. He asked what types of controls exist for those situations?

Mr. Bushel responded that they ask all the scientists to upload the experimental information and data in its entirety. Existing problems in many of the developed systems include the fact that they have tracked only some of the information and failed to carry along experimental information about a particular project or other quality control or assurance information. He set out to build maps as an experimental or project system and incorporated that with the array database to allow a query of the particular experiment or cross particular experiments. In-house information is all encompassing and includes all data and experimental information from the investigator and the investigator's contact person; also included is the process of the particular hybridization, whether it was a floor flip or some other process along that line, but this aspect is currently a major issue. Much of the informatics and the scientists involved in this technology are deciding on what you use to upload into a global database. Should one upload all the statistical data or just upload ratio values? Maps will allow one to query all the ratio data and ArrayDB will accept the whole output, but it is based on the platform used in-house so the challenge becomes the identification of a standard for each different platform. A type of standard is needed that can be utilized to normalize the expression data across all these platforms, yielding data analysis in one form type.

Dr. Stitzel commented that, with regard to quality assurance (QA) issues, this data entry process is not going to be Good Laboratory Practice (GLP) compliant because all investigators will be entering the data; she suggested that the investigators must be under the scrutiny of QA to ensure that the data are entered correctly. Even more importantly, the Food and Drug Administration (FDA) electronic data standards are just one example of a possible dilemma because they don't want us to change our electronic records. Further, change to electronic systems must be validated.

Mr. Buschel responded that he is attempting to keep the Center and the lab from modifying data or the information in any way and have a direct upload. As soon as the information comes out of the scanner or the computer it would be uploaded right into the database. Although some information must be entered in manually, he would like to limit it as much as possible.

Dr. Merrick responded that his group has put into place a QA/QC procedure where three individuals are responsible for monitoring experiments and are quality controlling the output prior to its upload into the repository database. They have a database where data are manipulated as the experiments are ongoing. They also intend to build a tox database repository for the Institute that will be accessible to the general public and QA/QC measures will be conducted with the data in the database.

Dr. Stitzel asked for public comment, and with none, she asked if there were additional comments from the Committee.

Dr. McClellan stated that he was impressed by the range of presentations and thought it was an excellent overview. He asked about the overall magnitude of the NIEHS effort in terms of the gene expression and proteomics activity.

Dr. Lucier responded that he was unable to provide a people and dollar value, but that various parts of the Institute are obviously working in this area. Support is also found through the NIEHS Extramural Program and those dollars are increasing as well. A substantial commitment to this project has been made through our traditional intramural research programs, through the NTP testing activities, and through the grants program, and he expects it to touch all major parts of our budget. The Small Business Innovative Research (SBIR) grants may be able to help with some of the methods development. We expect this aspect to grow and become very collaborative in nature. The project is not just focused in one single group, but rather is intertwined throughout parts of the institute and through our collaborators outside the institute as well. He stated that there is strong interest and commitment from the NIEHS and the NTP.

Dr. Hurt stated that the presentations have proposed an exciting vision of a normal human being on microchips that would eventually replace the whole animal for toxicology testing. She asked if, upon realization that the science of toxicology deals with detecting unintended and unexpected side effects, is this vision theoretically possible, and if so, is it timely to begin planning the strategy of how we achieve this goal?

Dr. Merrick responded that the goal is to eventually be able to look at human expression patterns and detect these effects. He stated that the ability to do that depends on the robustness of data sets and that there is still much work to achieve extrapolation to model systems for humans.

Dr. Stitzel agreed with Dr. Hurt that in the future, toxicology should change from looking at what appears in an animal to knowing what would occur in a human. She suggested that we start thinking about how we are going to move forward due to these changing viewpoints. This movement will require a strategy that allows one to predict occurrences in the whole organism.

Dr. Curren noted that most of the information presented today, certainly all for humans, was based on transformed cell lines. Further, most of the animal data resulted from transformed cell lines other than the data that came from the liver. He asked how this would compare with normal cells?

Mr. Buschel responded that all model systems have limitations, and while some information can be gained from the transformed cell lines, obviously primary human cell lines and primary rodent cell lines would be ideal. The limitations of each of the systems must be understood and one can still gain information, but it does cause concern.

Dr. Theran asked if it is anticipated that assays resulting from these tools will come to the NTP Center (NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, NICEATM) for validation?

Dr. Lucier responded that although these systems are in a very early stage, eventually they should move to the NTP Center for validation.

Dr. Stitzel commented on the differences in the databases regarding aspects such as validation of a method to achieve the same response, inter- and intra-laboratory reproducibility, etc.

Dr. Theran asked if, considering EPA's program to assess environmental toxins, there might be an opportunity to apply some of these tools.

Dr. Stokes responded that with regard to the High Production Volume (HPV) program, microarray systems will be addressed at the workshop on *In Vitro* Methods for Assessing Toxicity and this technology might be applied to achieve more accurate predictions from these systems. He stated that he expects to see the application of some of this technology to increase the accuracy of existing methods and that is how Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) will likely encounter it initially. Additionally, workshops and expert panel reviews of methods currently in some stage of development and validation are convened and the experts will be asked if these technologies can improve predictions.

Dr. Stitzel thanked Dr. Paules for organizing the helpful and impressive afternoon session.

Adjourn:
The day was concluded with a discussion by ACATM regarding validation considerations during methods development and the opportunity for public comment. The meeting was adjourned at 5:15 p.m.

Wednesday, March 8, 2000

I. Welcome and Introduction

Dr. Katherine Stitzel, Chair of ACATM, called the meeting to order at 9:55 a.m. and asked everyone to state his/her name and affiliation for the record.

Dr. John Bucher, Deputy Director, ETP, welcomed everyone to the meeting and explained that Dr. Mary Wolfe and Dr. George Lucier were unable to attend due to schedule conflicts.

Dr. Bucher explained that this meeting would include updates on the current activities of ICCVAM, the regulatory status of the Local Lymph Node Assay (LLNA) and Corrositex®, and the role of ICCVAM in test method development and validation. He announced that there

would be an addition to the agenda, as Dr. Schechtman would be presenting a summary of his recent participation in a European Center for the Validation of Alternative Methods (ECVAM) workshop. Dr. Bucher concluded by emphasizing that NIEHS and the NTP would maintain a strong commitment to alternative test methods.

II. Update on NTP Center and ICCVAM Activities

Dr. William S. Stokes, Director of NICEATM at NIEHS, provided an update on recent activities of the NTP Center and the ICCVAM. He began by reviewing the status of the LLNA and Corrositex® assays, stating that the final reports had been completed in February 1999 and June 1999, respectively. Regulatory acceptance was announced by agencies in October 1999 and the LLNA, a test method for assessing the allergic contact dermatitis potential of chemicals, is expected to be considered as a draft test guideline later this year by the Organization for Economic Cooperation and Development (OECD). Statements indicating regulatory acceptance of Corrositex®, an *in vitro* test method for assessing the dermal corrosivity potential of chemicals, have been received from the Consumer Product Safety Commission (CPSC), the Occupational Safety and Health Administration (OSHA), FDA, and Environmental Protection Agency (EPA). Additionally, the U.S. Department of Transportation (DOT) continues to accept the use of Corrositex® for determining packing group status.

Dr. Stokes then discussed the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX), which is proposed as a screening method to detect the developmental toxicity potential of chemicals. The assay evaluates the following endpoints:

- mortality, which is reported as the lethal concentration inducing death in 50% of exposed embryos (LC50);
- growth retardation, which is reported as the minimum concentration to inhibit growth (MCIG);
- malformations, which are used to calculate the effective concentration inducing malformations in 50% of exposed embryos (EC50); and the
- Teratogenic Index (TI), which is calculated as the LC50 divided by the EC50.

Advantages of the FETAX system are that it is relatively rapid and inexpensive. He explained that the current decision criteria for positive results are a TI value greater than 1.5 and an MCIG/LC50 ratio of less than 0.3. The current FETAX database includes FETAX data on 137 substances evaluated in 276 tests. Thirty-five of those substances were also tested with a metabolic activation system, consisting of activated rat liver microsomes. Rat, mouse, and/or rabbit reference data were located for 90 of the 137 substances. Human data, from human epidemiology and case reports, were available for 31 of the 137 substances. An interlaboratory study has been completed, with 26 chemicals tested in three to six labs. An ICCVAM Developmental Toxicity Working Group (DTWG), chaired by Dr. Angela Auletta at the U.S. EPA, has worked with the NTP Center to organize the upcoming expert panel meeting on FETAX.

Dr. Stokes then discussed the upcoming ICCVAM/NICEATM Expert Panel Meeting on FETAX, scheduled for May 16-18, 2000, in Research Triangle Park, NC. Drs. George Daston and Elaine Faustman will chair the Panel. There will be five breakout groups as follows: Environmental Breakout Group, Performance Breakout Group, Protocol Breakout Group, Reliability Breakout Group, and R&D Breakout Group. A total of 43 expert scientists from the U.S. and five other countries will serve on the breakout groups. The meeting will include an introductory plenary session, a series of breakout sessions, and a closing plenary session, all of which will be open to the public. Dr. Stokes announced that a comprehensive background review document prepared by the NTP Center would be available to the public prior to the meeting. The objectives of the meeting are as follows:

- To develop a consensus on the current validation status of FETAX, including its demonstrated accuracy and reproducibility;
- To develop a consensus on the current and potential usefulness of the assay for specific purposes to include:
  - Screening chemicals/mixtures for hazard potential
  - Prioritizing chemicals for further testing
  - Evaluating hazard potential of environmental samples (e.g., surface and ground water)
  - Weight of evidence evaluations of human developmental toxicity hazards;
- To identify research and development efforts that might improve the accuracy and reproducibility of FETAX; and
- To identify validation studies that would further characterize the usefulness and limitations of FETAX.

Dr. Stokes next discussed the upcoming ICCVAM Peer Review meeting for the Up-and-Down Procedure (UDP), an alternative method for assessing the acute oral toxicity potential of chemicals. He provided background information on the assay, explaining that it was adopted as Test Guideline 425 by OECD in 1998, as an alternative to the traditional acute oral toxicity method, Test Guideline 401. However, as a result of harmonization of classification criteria for acute toxicity and the proposed deletion of traditional test guideline 401, revisions to the UDP TG425 were necessary. An U.S. EPA Task Force subsequently prepared a revised UDP that also includes a revised limit test procedure and an optional supplemental test that generates slope and confidence interval data. He explained that the ICCVAM established an Acute Toxicity Working Group (ATWG) following a request from U.S. regulatory agencies to assess the validity of the revised UDP as a replacement for Test Guideline 401. He provided a list of ATWG members and a tentative timeline for the peer review of the UDP. The peer review meeting is currently scheduled for July 25, 2000.

Dr. Stokes next discussed the ICCVAM assessment of the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC). A preliminary review concluded that the MEIC data suggest that *in vitro* methods might potentially be useful in estimating acute toxicity. Based on this premise, an expert workshop has been recommended to review the MEIC approach and other *in vitro* approaches and to identify future efforts needed to validate promising methods. The MEIC studies were completed in 1996 and published in 10 different publications between 1998 and 2000. The purpose of these studies was to investigate the relevance of *in vitro* tests for predicting the acute toxicity of chemicals in humans and to identify batteries of existing *in vitro* tests as replacements for acute toxicity tests using animals. The project involved 96 laboratories testing 50 reference chemicals using 82 *in vitro* assays. The *in vitro* IC50 values (i.e., that dose estimated to cause a 50% decrease in some measure of growth or cell viability) were compared to estimated human blood 50% lethal concentration (LC50) values. Based on an analysis of the data collected, it was determined that a battery of three human cell line tests was highly predictive of human blood LC50 values, with a r^2 of 0.77. Tentative topics for the upcoming workshop included:

- Cytotoxicity test methods,
- *In vitro* strategies to support selection of appropriate *in vivo* studies,
- Toxicokinetic assessments, and
- Reference chemicals suitable for validation studies.

Dr. Stokes next discussed test methods that may be reviewed in the future. Transgenic mouse carcinogenicity assays and selected endocrine disrupter screening methods are expected to be scheduled for peer review in 2001/2002. He explained that two transgenic mouse carcinogenicity assays, the TG.AC and the p53+/-, have been proposed as possible substitutes for the 2-year mouse bioassay and that a Background Review Document (BRD) will be prepared by the NTP ETP and NICEATM. The endocrine disrupter methods include *in vitro* receptor

binding assays and transcriptional activation assays to detect androgen and estrogen activities. A BRD will be prepared by NICEATM.

Dr. Stokes concluded his presentation by providing a list of current OECD-related activities involving ICCVAM that have involved ICCVAM working groups or that he has been involved with. These include the 3T3 Neutral Red Uptake (NRU) phototoxicity test; the rat skin Transcutaneous Electrical Resistance (TER) and Episkin test methods for evaluating corrosivity; a guidance document on humane endpoints for animals used in toxicological studies; and a guidance document on validation principles and criteria for evaluating the validation status of new methods.

Discussion:

Dr. Susan Hurt stated that she hoped, with respect to the OECD guidance documents, that ICCVAM would make a serious effort to respond to the OECD's request for comments. Dr. Stokes stated that comments would indeed be provided. Dr. Hurt continued by commenting that working within regulatory agency time restraints was important and that she was pleased to see the effort to accommodate the U.S. EPA's schedule for the review of the Up-and-Down procedure.

Dr. Rodger Curren asked if the entire ICCVAM panel was receiving the official OECD documents that are distributed. Dr. Stokes stated that NICEATM would distribute this information to all panel members. Dr. Curren then inquired as to whether it would be appropriate for the ICCVAM panel as a whole to make a recommendation to the OECD. Dr. Stokes replied that, currently, only individual comments were submitted as the request for comments was sent to individuals who appear on a contact list. Dr. Stitzel suggested submitting an ICCVAM statement in addition to individual comments. Dr. Stitzel stated that this kind of comment from ICCVAM would be particularly useful for those methods that were reviewed by ICCVAM.

Dr. Peter Theran stated that he was pleased to see that the U.S. EPA Endocrine Disrupter program is sending its *in vitro* tests through ICCVAM, but that he was concerned that they do not appear to be forwarding the *in vivo* tests in a similar manner. He stated that *in vivo* tests needed to be subjected to a higher level of scrutiny. Dr. Stokes replied that the decision to initially forward *in vitro* methods was based on limitations of resources and the stage of validation for the various methods. The U.S. EPA felt that the *in vitro* methods were further along in the standardization and validation process, and therefore, should be first in the review process. He added that the U.S. EPA does not currently have the resources to prepare BRD and has requested that NICEATM prepare this information until a contract is in place to carry out this work.

Dr. Alan Goldberg stated that if ICCVAM is to serve as a final pathway for similar public programs, then the Committee needs to make a stand with respect to *in vitro* and *in vivo* methods, and questioned whether ACATM should make a statement regarding this matter. Dr. Stitzel asked if Dr. Goldberg wished to make a motion to that effect and he responded by making a motion that the ACATM make a public statement to the U.S. EPA regarding this issue. Dr. Theran seconded the motion, which read as follows:

> *The National Toxicology Program Advisory Committee on Alternative Toxicological Methods (ACATM) believes that the status of in vitro and in vivo methods used for regulatory toxicological needs should be evaluated by the ICCVAM process. In the case of the endocrine disruptor program in development, ACATM is concerned that only in vitro methods will be submitted to ICCVAM for review. ACATM requests (by unanimous voice vote) that all methods under consideration for endocrine disrupter testing be submitted for*

evaluation. ACATM will work with ICCVAM to facilitate review of all submissions in a timely fashion. If the U.S. EPA is unable to provide the necessary material in the time sensitive period, or if the methods are reviewed and are not considered valid, then implementation of the testing should be delayed until scientific peer review and validation can be accomplished.

ACATM also recommends that interaction between ICCVAM and the U.S. EPA Science Advisory Panel and Science Advisory Board take place to ensure a coordinated validation effort.

Dr. Rodger Curren stated that he understood the wording in the original ICCVAM report to imply that ICCVAM would only act on those assays that affect multiple agencies. He questioned whether the U.S. EPA's screening and testing methods would be acceptable under this wording. Dr. Stokes replied that the original intent of ICCVAM was to consider test methods of multi-agency interest, but that this would not preclude other methods from being considered. He added that while the U.S. EPA was charged by law to develop and validate these methods, endocrine screening and testing methods are of interest to multiple agencies. Dr. Katherine Stitzel asked if the panel was ready to vote on the motion. Dr. Bucher read the conflict of interest statement with respect to voting. The motion was passed by a unanimous vote.

Dr. Sidney Green asked for clarification as to what part of the acute toxicity methods, endorsed by OECD, required revision. Dr. Stokes replied that revisions were needed because hazard classification categories were different between countries with respect to the estimated LD50 range of values for each category. A classification scheme has now been harmonized among OECD member countries and includes five hazard categories based on the estimated LD50: < 5mg/kg; 5-50 mg/kg; 50-300 mg/kg; 300-2000 mg/kg; and 2000-5000 mg/kg.
Dr. Stitzel added that the fixed dose and acute toxic class methods were based on European standards with an upper limit dose of 2000 mg/kg. This upper dose did not meet the current needs of U.S. regulatory agencies, which require information at doses up to 5000 mg/kg.

Dr. Green asked about the identity of the four transgenic mouse models being evaluated by the International Life Science Institute (ILSI). Dr. Bucher replied that four models were the Tg.AC mouse, the heterozygous p53 knockout mouse, the XPA repair-deficient mouse, and the H-ras2 mouse. In addition, the neonatal mouse and the *in vitro* SHE cell assay are also being evaluated.

Dr. Montgomery asked whether NICEATM had adequate financial and logistical support for all of its endeavors. Dr. Stokes replied that NICEATM was currently operating at maximum capacity and that consideration of any new submissions would need to be deferred. He added that a critical prerequisite for review of a test method is organizing all of the supporting information in a manner that makes the review as convenient as possible for panel members. As a result, the greatest amount of the Center's time and resources are spent on this process. Dr. Curren asked if the current ICCVAM Authorization Bill before Congress would be of any assistance in better defining resources and funding. Dr. Bucher stated that this bill could allow Congress to appropriate funds for NICEATM activities.

Dr. Alan Goldberg asked if he was correct in his understanding that the 3T3 NRU and the two additional *in vitro* corrosivity assays that were approved by ECVAM are being submitted to OECD prior to review by ICCVAM. Dr. Stokes replied in the affirmative, stating that the 3T3 NRU and the two corrosivity methods have been adopted by the European Commission (EC) and that the EC has now submitted these methods to the OECD for international adoption.
Dr. Goldberg then asked if there was an established review process for handling OECD guidelines that are presented to the United States. Dr. Stokes replied that when an OECD guideline is forwarded to the U.S. for comment, the EPA solicits comments and prepares an U.S. National position. No formal peer review is conducted as the OECD is merely striving to gain

international consensus on a general protocol for a test method and fails to evaluate the extent that an assay protocol has been validated. Dr. Goldberg suggested that a peer review mechanism for those assays approved by ECVAM should be explored. Dr. Stokes responded that an ICCVAM Working Group did prepare comments on the two OECD *in vitro* corrosivity methods. Dr. Rowan stated that he was aware that the transgenic models are considered to be refinements and asked if the issue of refinement will be addressed when those models are reviewed. Dr. Stokes responded that the submission guidelines require this aspect to be addressed in the background documentation and that this information is specifically reviewed by the panel.

III. Regulatory Acceptance Status of ICCVAM-Recommended Methods: Corrositex® and LLNA

A. U.S. EPA - Via telephone, Dr. Karen Hamernik, U.S. EPA, provided an overview of the U.S. EPA's response to ICCVAM recommendations concerning Corrositex® and the LLNA. She stated a standing EPA intra-agency committee, which has representatives from various organizational components of the EPA, has been formed to evaluate and make comments and recommendations on ICCVAM reports and products. U.S. EPA members of ICCVAM provide the committee with general information about the method or methods under consideration and the ICCVAM review. The viewpoints of the various intra-agency committee participants are coalesced into a response letter that is sent back to ICCVAM.

With respect to the status of the LLNA and Corrositex®, Dr. Hamernik explained that the U.S. EPA has announced acceptance of both methods. Associated stipulations are discussed in the response letter. The Agency is currently undertaking a program follow-up, test guideline review and revision incorporating the LLNA and Corrositex, and guidance development. In the near future, the Agency plans to present the test guideline revisions to the SAP (Science Advisory Panel) for comment by that group, the public, and other interested parties. She said that the U.S. EPA will inform the public and regulated community regarding assay acceptance via press releases or Federal Register notices and possibly via a future workshop. An EPA website is under development that will provide information about what EPA is doing with regard to alternative toxicological test methods. Finalized test guideline revisions would be eventually posted on the test guideline website (http://www.epa.gov/epahome/standards.html).

Dr. Hurt stated that she was pleased to see the progress of the U.S. EPA. She mentioned aspects stated in Dr. Galson's letter that would need to be addressed prior to implementation of the LLNA, and asked if there was any assistance that ICCVAM or NICEATM could provide to help foster the review process within EPA. Dr. Hamernik replied that an in-house EPA workgroup is fine-tuning the points addressed in the letter and that there may be situations where the assay is not applicable. She said that the LLNA is new for many of the scientists involved, but that they have been in contact with scientists from Proctor and Gamble who have been using the assay and that the exchange of information has been very helpful. She stated that at this time, they are progressing towards the SAP and are identifying scientific and possible regulatory issues.

Dr. Stitzel asked if a date has been set for the SAP meeting. Dr. Hamernik replied that the meeting is tentatively scheduled for September.

Dr. Green asked if Dr. Hamernik could provide more information as to the nature of the follow-up activities being performed by the in-house EPA workgroup with respect to Corrositexâ and the LLNA. Dr. Hamernik replied that these activities include drafting revisions to existing EPA test guidelines, discussing EPA-specific regulatory and scientific issues, discussing the strengths of the assays as compared to current procedures involving animal tests, and implementing the plan for incorporating these assays.

Dr. Hurt asked if the September SAP meeting would focus on these guidelines specifically or if it would be a general review of toxicity testing guidelines. Dr. Hamernik replied that the two assays and their guideline revisions would be the only test guideline topics.

B. FDA - Dr. Leonard Schechtman, U.S. FDA, gave an update on FDA's process for considering ICCVAM-recommended methods. He reviewed the structure of the FDA, where, in addition to ORA (Office of Regulatory Affairs) and NCTR (National Center for Toxicological Research) there are five product Centers with different regulatory perspectives, mandates, and roles that are all part of the FDA. With regard to the FDA process for addressing ICCVAM recommendations, Dr. Schechtman stated that following ICCVAM peer review, the ICCVAM recommendations are reviewed by each of the FDA Centers, whereupon each Center submits comments regarding test method acceptability and applicability. He added that the applicability of ICCVAM-recommended tests is a Center-by-Center decision and that each Center uses different methods and criteria to determine such applicability. The decision to implement a test is based on whether the method satisfies a Center's scientific criteria in meeting its regulatory commitment to establish product safety.

Dr. Schechtman then reviewed the FDA's response to the ICCVAM recommendations on the LLNA. The FDA feels that the LLNA is an acceptable alternative to the Guinea Pig Maximization Test (GPMT) for hazard identification of strong and moderate contact chemical sensitizing agents. The FDA acknowledges the assay limitations expressed by the ICCVAM peer review panel and the Immunotoxicity Working Group (IWG) that would necessitate the use of the traditional GPMT in some instances. FDA also recognizes the animal welfare advantages of using the LLNA, such as animal use refinement and potential animal use reduction.

Dr. Schechtman then described in more detail the acceptability and applicability of the LLNA within FDA. He stated that despite FDA's general acceptance of the LLNA as a validated alternative to the GPMT, the application of the LLNA varies from Center to Center:

- The Center for Drug Evaluation and Research (CDER) has concluded that the LLNA is an acceptable stand-alone alternative to the GPMT for hazard identification of dermally applied contact sensitizing agents within the limitations acknowledged by the ICCVAM peer review panel. CDER recognizes certain advantages of the LLNA as compared to the GPMT, such as refinement in animal use.
- The Center for Biologics Evaluation and Research (CBER) concluded that the LLNA is an acceptable alternative to the GPMT. CBER proposes to recommend the LLNA when skin sensitization data are necessary.
- The Center for Veterinary Medicine (CVM) concluded that the LLNA is an acceptable alternative, but that CVM has no need to include the method in its testing guidelines. On the rare occasions when a veterinary drug may be tested for hypersensitivity potential, preference is given to the use of the most appropriate test animal.
- The Center for Devices and Radiological Health (CDRH) concluded that the LLNA is acceptable with qualification. Examples of qualifications include that the LLNA is inappropriate for testing metal salts, proper positive and negative controls for testing extracts of materials/devices need to be included in the testing structure, and it is recommended that both auricular lymph nodes per mouse should be tested.
- The Center for Food Safety and Applied Nutrition (CFSAN) concluded that the LLNA is a valid, stand-alone alternative to the GPMT for detecting allergic contact dermatitis (ACD) mediated by food and color additives and cosmetics, within the limitations expressed by the ICCVAM IWG. Acceptance of the test means that the LLNA can be substituted where the GPMT would otherwise be used to assess ACD potential of a compound regulated by CFSAN.

Dr. Schechtman explained that the notification process for Center regulatory units involves educating the regulatory review staff through internal seminars, workshops, and training courses. Participation in meetings, conferences, and panels that include information on the scientific

basis and use of a test is a means of gaining familiarity with new test methods. He also noted that scientific publications and Internet websites help to inform FDA's regulatory review staff of the availability and acceptance of certain tests by a particular Center. With regard to notification of the public and regulated industry, Dr. Schechtman stated that the process involves publicizing the anticipated use of the new test method as it may apply to a Center's testing prescripts and/or regulatory guidance. The availability and application of an ICCVAM-recommended method is communicated via publications, presentations at open meetings, guidance documents, guidelines, regulations, Federal Register notices, and Internet websites.

Dr. Schechtman also reviewed the FDA response to the ICCVAM recommendations regarding Corrositex®. He stated that the FDA product centers, except for CFSAN, do not assess the dermal corrosivity potential of the products they regulate; therefore, there is no anticipated use for Corrositex® with the possible exception of CFSAN. The acceptability and applicability of Corrositex® for use by CFSAN is pending further evaluation. Dr. Schechtman stated that CFSAN anticipates eventual affirmation of the scientific validity of Corrositex® for the very limited classes of compounds recommended by ICCVAM (i.e., acids, bases, and acid derivatives).

Discussion:

Dr. Theran asked Dr. Schechtman to comment on how having a member(s) from each Center involved in the ICCVAM process is helpful with facilitating the response within FDA. Dr. Schechtman replied that this level of participation is both beneficial and effective. He stated that input from each of the FDA Centers and ORA are essential to achieving FDA-wide, cross-center positions on ICCVAM issues. He explained that as a FDA spokesperson, it was his job to coordinate and compile comments and recommendations from the various Centers/ORA and to develop Agency-level positions that accurately communicate the regulatory needs of the FDA in evaluating, accepting, and implementing ICCVAM-recommended methods.

Dr. Goldberg asked if the FDA has independently evaluated the 3T3 NRU assay, which has already been validated by ECVAM, and is being submitted to OECD prior to consideration by ICCVAM. Dr. Schechtman replied in the affirmative, stating that the FDA is participating in phototoxicity workgroup efforts, has its own phototoxicity team that is examining proposed methods, and has been involved in international efforts through the International Conference on Harmonization (ICH).

Dr. Stitzel stated that the International Life Sciences Institute's (ILSI) training task force is willing to conduct workshops or training sessions for the FDA. Dr. Schechtman stated that the Centers would welcome this opportunity. Dr. Curren stated that the Institute of In Vitro Sciences, Inc. would also be willing to assist the FDA with training. Dr. Schechtman commented that it was first thought that the training would come from the test developers but that these additional options would be welcomed.

Dr. Andrew Rowan asked if, with respect to the LLNA, the FDA is stating that either the LLNA or the Guinea Pig test may be used. Dr. Schechtman stated that there had been no ruling to totally replace the Guinea Pig test, but that the FDA recognizes the LLNA as a valid alternative test and that sponsors would be encouraged to use it when appropriate and acceptable. He added that those considerations should be clarified in advance with the particular FDA product Center to which such data will be submitted.

Dr. Stitzel commented that she was aware that there has been some difficulty in obtaining the strain of mouse used in the LLNA, thereby indicating increasing use of the assay.

IV. Summary of Revisions to ICCVAM Test Method Submission Guidelines

Dr. Stokes presented a summary of the revisions to the ICCVAM test method submission guidelines. He began by providing the history of the guidelines, which were originally developed by the Corrosivity Working Group (CWG), in 1996 in response to a test method submission referred to the *ad hoc* ICCVAM. An *ad hoc* working group was convened in 1997 to further develop the initial general submission guidelines, which were subsequently published in May 1998. Test sponsors used this version of the guidelines for preparing the LLNA and Corrositex® submissions. The guidelines were revised in October 1999 (NIH Publication No. 99-4496) and endorsed by ICCVAM at the February 29, 2000 meeting. He explained that comments from this ACATM meeting would also be incorporated into the revised guidelines.

Dr. Stokes then discussed the purpose of the revised submission guidelines and provided an overview of each of the sections. He concluded by describing additions that had not yet been incorporated into the document, but that would be in the final version of the guidelines. These include an introduction to Section 1, a description of the training requirements in Section 11, additional references, and instructions to append the test method protocol.

V. Committee Discussion/Recommendations on Submission Guidelines

Dr. Theran asked if the submission guidelines would be affected by recommendations from ACATM. Dr. Stokes replied in the affirmative, stating that if the recommendations are relevant, then they will be considered and incorporated where appropriate.

Dr. Goldberg informed the committee that the Johns Hopkins Center for Alternatives to Animal Testing has received funding from the National Institutes of Health (NIH) to develop a search engine to search databases for information on alternative methods. He stated that they have been working in conjunction with Alternatives to Animal Testing on the Web (ALTWEB), Fund for the Replacement of Animals in Medical Experiments (FRAME), and the National German Centre for the Documentation and Evaluation of Alternatives to Testing in Animals (ZEBET), and within two weeks, a beta version of the search engine would be available. Dr. Goldberg added that they plan to develop specialized database search engines as well, and this technology should be readily available to the general public within six months.

Dr. Roger McClellan asked Dr. Stokes to provide an overview of the efforts to make the guidelines broadly available to the scientific community. He asked how many copies had been distributed, especially with respect to those individuals that are not directly in touch with the scientific community. Dr. Stokes replied that the guidelines are available on the Internet, but that he did not have a firm figure for the number of hits received. He said that the guidelines had also been distributed via a set list of contact organizations established by NICEATM. Dr. McClellan suggested submitting a brief summary to various groups and organizations for inclusions in newsletters and journals.

Dr. Ray Tice stated that the guidelines and other information regarding ICCVAM would be available at the NTP booth at the upcoming Society of Toxicology (SOT) annual meeting. Dr. Stokes added that there is an ICCVAM list-serve available to the public that automatically forwards press releases, Federal Register notices, and all other pertinent information relating to ICCVAM activities.

Dr. Kenneth Ramos suggested that the guidelines include a list of abbreviations at the beginning of the document. He mentioned that under Section 3 of the revised guidelines, the statement is made that physical and chemical properties should be included "to the extent possible". He questioned whether this statement was adequate as he felt that this is a critical issue for validation studies and should be more strictly emphasized. Dr. Stitzel replied that, for human

and older animal studies, such information may be limited and all that can be provided is the available information. Dr. Wallace Hayes added that this is also a problem with formulated products. Dr. Stokes agreed that the guidelines should emphasize the importance of providing this information wherever possible.

Dr. Green stated that on page I-3, some of the persons listed as members of the ICCVAM committee have retired and suggested that the list be updated. Dr. Stokes replied that a revised version of the list would be included in the next version of the guidelines. Dr. Hayes questioned the necessity of including the list of ICCVAM members in the guidelines. Dr. Stokes replied that the member agencies and persons involved should to be identified and credited. Dr. Stitzel suggested adding the statement that the list was current as of the date printed.

Dr. Green urged that adherence to the spirit of Good Laboratory Practices (GLPs) needs to be one of the general principles stated early in the document. Dr. Montgomery commented that this statement could be dangerous because GLPs are loosely interpreted at times. Dr. Stitzel suggested incorporating a definition of GLPs into the document. Dr. Green then commented that Section 2.1.15 was vague, as was the word “call” on page B1, and both should be clarified, and he further questioned the use of the word "all" in the statement “include all data” in Section 9.1. Dr. Stokes suggested changing the wording to state “all available.” Dr. Green suggested “all relevant.” Dr. Schechtman stated that the original intent was to avoid filtering out data. Dr. Stitzel suggested that a written summary of all types of data available as well as rationale for why the submitted data were selected should be included with the submission.

Dr. Hurt stated that, with respect to Section 2.0, the word “protocol” has a specific meaning under the GLPs and that she was unclear as to whether the use of the word refers to test guideline, protocol, or something between the two. She asked if it would be possible to include a sample protocol in an appendix to the submission guidelines. There was general discussion about this issue, but no consensus as to the appropriateness or need for a sample protocol or guideline in an appendix.

Dr. Stitzel stated that, with respect to the OECD process for accepting test methods, a test method is submitted to the OECD with no information as to how the proposed method was validated or whether ICCVAM and/or ECVAM have approved the method. She suggested that the OECD should develop a procedure for indicating that a test method has been validated. Dr. Stokes replied that the international review process would be expedited if appropriate documentation of validation was submitted with the test method and provided to all OECD member countries. Dr. Stitzel clarified that she was suggesting that the OECD review process be different for validated methods as a means to indicate that agreement exists among member countries that the validation process is meaningful.

VI. ECVAM Workshop on Novel Pyrogen Tests Based on the Human Fever Reaction

Dr. Schechtman gave a brief presentation on the ECVAM Workshop on Novel Pyrogen Tests based on the human fever reaction, that he attended in Konstanz, Germany on January 16-20, 2000. He first explained the overall workshop objective, which was to identify *in vitro* methods that could serve to replace the currently utilized *in vivo* rabbit pyrogenicity test. He added that the *in vitro* Limulus amoebocyte lysate (LAL) assay for gram-negative endotoxins has already reduced animal usage for pyrogen testing by approximately 80%. The tests under consideration would be complementary to the LAL, but would be sensitive to gram-positive endotoxins and non-endotoxin pyrogens, such as cytokine (interleukin) release tests using whole human blood (WHB). The goal of the workshop would be the initiation of pre-validation and validation

studies for candidate tests (i.e., WHB/IL-6). Dr. Schechtman then described the WHB cytokine release test, and outlined the specific workshop aims:

- To bring together the primary experts in the pyrogen testing arena, with special emphasis on *in vitro* expertise;
- To identify the most promising alternative pyrogen tests;
- To review the applicability and spectrum of responsiveness of those tests;
- To develop a collaborative research effort for the purpose of addressing pre-validation/validation issues; and
- To define the needs and steps toward validation and regulatory acceptance of such alternative methods.

Dr. Schechtman stated the FDA's objectives for participation in the workshop. These included gaining knowledge of the state-of-the-art tests for pyrogenicity and development of alternative/*in vitro* tests for pyrogens. It also provided the opportunity to contribute a U.S. regulatory perspective regarding test development, pre-validation/validation, acceptance, implementation, and communication. A third objective was to help bridge the communication and interaction between ICCVAM and ECVAM, which are sister organizations with similar goals. Lastly, FDA wished to observe/experience the workshop process utilized by ECVAM in order to ascertain similarities/differences with ICCVAM and whether the ECVAM process can or should be used by ICCVAM.

Dr. Schechtman observed that ECVAM becomes engaged in the pre-validation and validation process at its earliest stages. He also noted that ECVAM provides procedural guidance and direction to test developers, researchers, and involved parties. He indicated that ECVAM appoints senior staff who navigates the workshop process. Considerations that Dr. Schechtman found to be noteworthy included ECVAM's financially solvent status. ECVAM finances directed research and collaborative trials essential to the pre-validation/validation process. ECVAM also finances and conducts its own research in the area of alternatives to whole-animal testing. To date, ECVAM has funded about 50 workshops, publishes its own journal (Alternatives to Laboratory Animals [ATLA]), and employs approximately 28 full-time staff.

Based on Dr. Schechtman's observations, he named a few aspirations that would be beneficial to the ICCVAM process. These included being more proactive in actively soliciting the scientific community for candidate tests; identifying and publicizing areas of regulatory testing that are in need of animal use refinement, reduction, and/or replacement; and funding research projects aimed at development and validation of alternative test methods.

Further, he also offered a FDA perspective on ICCVAM's limitations. ICCVAM's financial support is about ten-fold less than that of ECVAM. Without sufficient financial resources and ability to fund research, test development, pre-validation, validation, Work Group, Peer Review Panel, and Steering Committee efforts, ICCVAM's influence, effectiveness, and rate of progress are greatly limited. Lastly, Dr. Schechtman felt that ICCVAM needs significant additional Federal financial backing to support its efforts in order to meet all of its objectives and reach its full potential.

Dr. Denison agreed with Dr. Schechtman that ICCVAM should identify and solicit tests for consideration. He added, however, that it was currently difficult to gain an understanding of the spectrum of tests, which are currently being used by different agencies, and that this knowledge was necessary in order to identify the opportunities for replacement, refinement, or reduction.

Dr. Hayes stated that he appreciated Dr. Schechtman's comments. He agreed that there is much work to be accomplished, but urged that people do not lose sight of the fact that ICCVAM funds were better spent on LLNA and Corrositex® than the three *in vitro* corrosivity assays that were

reviewed by ECVAM. He added that the dollar differential is not as great as it may seem upon initial review; further, an influx of dollars at this point would not make a difference in the amount of work that can be completed. Dr. Stokes replied that any initial additional funding received would likely be used to hire additional staff to accomplish high priority activities. He stated that he supports the suggestion of posting all of the methods used by regulatory agencies on a website, adding that it has been difficult at times to obtain this information.

Dr. Curren stated that the proposed ICCVAM Authorization Act has a component that states that all regulatory agencies will be required to provide information regarding the testing information they require for data submission. He stated that it should be noted that Corrositex® could not have been validated without the funding and effort of ECVAM and that he believes that the money that is filtered down for actual use by ECVAM is not as much as it may seem. He also stated that ECVAM has taken a proactive approach towards moving assays forward in validation, which is currently not the approach of ICCVAM. He questioned whether the ACATM should suggest a more proactive role for ICCVAM.

Dr. Rowan stated that there has been a focus on acute tests due to political pressure in the 1980s, but that there is a need to establish future priorities. He added that an assessment of regulatory requirements is needed to determine what methods are required for regulatory submissions and how many animals are being used for each method.

Dr. McClellan commented that he has not seen enough information to be able to draw conclusions regarding budgeting and staffing. He said that more analysis and information needs to be compiled before this issue could be discussed appropriately. Dr. Montgomery disagreed, stating that ICCVAM is one of the most productive government projects that he has participated in and that there is a need to be proactive with ICCVAM. He added that he felt that the NTP and NIEHS have done a remarkable job with limited resources. Dr. McClellan reiterated that he is not able to make an informed statement regarding financial concerns without all of the necessary information. Dr. Montgomery stated that it is necessary to plan ahead when Congress is involved and Dr. McClellan agreed. Dr. Goldberg commented that he agreed with both Dr. McClellan and Dr. Montgomery, adding that some of the major differences between ICCVAM and ECVAM are political as well. He said that these are very different political systems with different levels of funding and that it cannot be accurately judged without all of the information.

VII. Public Comment

Ms. Sarah Amundson from the Doris Day League provided a statement on behalf of People for the Ethical Treatment of Animals (PETA). She stated that she was pleased with the discussions in the meeting thus far and that the committee was dealing with issues of major concern. She stated that Dr. Stokes was instrumental in the success of ICCVAM as well as in effectively addressing public concerns. Ms. Amundson stated that the new validation and regulatory acceptance criteria contain a section specifically dealing with animal welfare concern, but pointed out that there is not a clear definition of the term "animal". She added that this term usually refers to those species that are included under the Animal Welfare Act (AWA) and that it should be noted that many toxicity tests use animals that are not included under the AWA.

Ms. Amundson then asked if any contact had been made with the USDA regarding the first two methods that were validated by ICCVAM, both of which replace species included under the AWA. Dr. Stokes replied that he had recently given a presentation to all of the U.S. Department of Agriculture (USDA) animal welfare inspectors and this presentation included an overview of these two test methods. He stated that he would notify USDA upon formal notification to industry by regulatory agencies that these methods have been accepted, adding

that part of the USDA mandate is to ensure that alternative methods have been adequately considered.

Ms. Amundson stated that she was pleased with Dr. Goldberg's move to make a statement to the U.S. EPA regarding their Endocrine Disrupter Program. She added that she was also pleased with the concern expressed by the ACATM for the need to place more effort on prioritization and increased interest in whole animal systems.

Ms. Amundson went on to say that she appreciated the information presented by the regulatory agencies and urged them to use the Federal Register to make their announcements, as this is a useful tool for animal protection groups. She added that if flexibility exists in the choice of whether or not to use an alternative method, members of industry would generally choose not to use the alternative methods.

Ms. Amundson concluded by mentioning the ICCVAM Authorization Act that has been introduced in the U.S. House of Representatives (HR4281) and the U.S. Senate (S1495). She stated that there is grave concern with respect to wording and that there needs to be awareness regarding the current resources going to ICCVAM. She said that without the appropriate wording for a dedicated funding stream, ICCVAM could miss out on some much needed funding. She stated that she would be contacting all of the ACATM members by mail asking for their support for this bill.

Dr. Bucher stated that, with respect to comments regarding funding and accessibility of information, the ACATM was established to review programs that have been put forth for review by ICCVAM and that the ACATM is not best-utilized dealing with funding issues.

VIII. Current ICCVAM/NICEATM Role in Test Method Development and Validation

Dr. Stokes presented information on the current role of ICCVAM and NICEATM in test method development and validation. He reviewed Public Law 103-43, which directed NIEHS to develop and validate assays and protocols for alternative methods that can reduce or eliminate the use of animals, to develop criteria for the validation and regulatory acceptance of alternative methods, and to develop a process to achieve the regulatory acceptance of scientifically validated methods. This legislation led to the formation of an *ad hoc* ICCVAM, which developed the document "Validation and Regulatory Acceptance of Toxicological Test Methods" (NIH Publication 97-3981, 1997). This document provided the ICCVAM criteria for validation and regulatory acceptance of alternative methods. He also discussed the process for the consideration of new test methods. He stated that the goal of ICCVAM and NICEATM is to promote the scientific validation and regulatory acceptance of new alternative test methods that are more predictive of human health and ecological effects than current methods, and methods that refine, reduce, and replace animal use where scientifically feasible.

Dr. Stokes explained that the current role of ICCVAM and NICEATM in test method development and validation consists of providing information and comments on proposed protocols/study designs to developers, evaluating test methods of multi-agency interest, convening expert panels and workshops to review methods, and providing recommendations to agencies. He then reviewed the ICCVAM test method evaluation process.

Dr. Stokes elaborated on the three types of meetings that are used to evaluate test methods. Workshops are used to evaluate the adequacy of current test methods, to identify toxicity endpoints for which improved test methods are needed, to identify promising methods that should undergo further development and validation, and to recommend appropriate validation studies, research, and model development needed to support new methods. Expert Panel

Meetings are convened to evaluate the status of test methods at various stages of validation, to recommend further research and model development efforts that may improve the test methods' performance, and to recommend additional validation studies necessary to further characterize the usefulness and limitations of a method. Peer Review Panel Meetings are convened to conduct a comprehensive review of all-available data and information for a test method, to evaluate the extent to which the ICCVAM validation and acceptance criteria have been addressed, and to develop a consensus on the usefulness and limitations of a method. The product of a peer review panel meeting is a published test method peer review report that is forwarded to Federal Agencies for their consideration.

Dr. Stokes concluded his presentation by discussing the driving forces for new test methods and new technologies that will impact toxicity testing. Significant advances in our understanding of the molecular mechanisms of toxicity and the opportunity to incorporate new science and technology are increasing efforts to develop new test methods. The desire for improved toxicity predictions, increased efficiency, and benefits to animal welfare and legislation are also influencing the development of new methods. He then listed new technologies that may impact how toxicity testing is conducted in the future. These include transgenics, toxicogomics, proteomics, molecular biomarkers, tissue engineering, high throughput technologies, and non-invasive imaging/labeling techniques.

Dr. Montgomery stated that he has some familiarity with 3-D computer models for mouse anatomy and fetal development and has found them to be quite useful. Dr. Stokes replied that this might be a good approach to apply to the FETAX evaluation of embryos.

IX. Current NIEHS Extramural Support of Test Method Development and Validation

Dr. Jerry Heindel provided an overview of the NIEHS extramural activities for alternative models. He stated that the extramural goals with respect to alternative models include developing alternative animal models and alternatives to using animals for toxicity testing, developing and validating models of disease, validating sentinel species, and developing alternative models that will facilitate the understanding of toxicant action and extrapolation to humans. He explained that the number of grants for alternative models has been steadily increasing over the past ten years, providing detailed information for 1998 where the extramural division supported 75 grants, 15 centers, and eight Superfund centers for a total of $7.3 million.

Dr. Heindel discussed the specific initiatives that have received support from extramural funding. In 1990, a program announcement (PA) was published regarding the development and utilization of transgenic animals and cell models in studies of environmental mutagenesis and associated health effects. Requests for Applications (RFA) were published in 1995 and 1996 for mechanistically based alternative models for toxicity testing and the use of transgenic model systems in molecular biology, respectively. The first of these RFAs funded 11 applications in the areas of developmental toxicity, immunotoxicity, cancer/mutations, biomarkers/assays, and computer databases. Grants funded as a result of this RFA included:

- *in vivo* and *in vitro* detection of carcinogen-induced gene deletions;
- DNA damage inducible genes as dosimeters for genotoxicants;
- an animal model of human hepatocyte carcinogenesis;
- p53 protein expression as a dosimeter of genotoxicity;
- shrimp assay for developmental toxicity;
- use of fish in immunotoxicity risk assessment;
- vitellogenin as a biomarker for estrogenic chemicals;
- *in vitro* cultures of human proximal tubules; and

- use of recombinant P450s for toxicology screening.

The objectives of the second RFA mentioned by Dr. Heindel focused on finding new transgenic models for identifying genes whose expression is altered by environmental agents in order to improve the relationship between exposure and disease, to investigate expression of DNA repair genes induced by xenobiotics, and to identify environmental agents that cause non-cancerous health effects. Grants funded as a result of this RFA included:

- transgenic models for assessment of neurotoxicant mechanisms and DNA repair;
- transgenic mouse models to study the role of platelet derived growth factor in lung fibrosis;
- response to injury in gamma glutamyl cycle deficient mice;
- heat shock proteins as modulators of developmental toxicity; and
- 3D visualization of anatomy and gene expression.

Dr. Heindel presented the numbers of alternative animal models funded by the Extramural Program. Some of these alternatives included 69 transgenic models, seven *Xenopus* models, five zebra fish models, five avian models, one *Drosophila* model, and various fish models. Of the transgenic models, seven were related to DNA repair, 15 focused on carcinogenicity/mutagenicity, 10 researched reproductive/developmental/endocrine toxicity, 16 focused on metabolism, and 13 studied specific toxicants. He then briefly described the Trans-NIH initiative in animal models, which include Trans-NIH Coordinating Committees for zebrafish and *Xenopus* and a Trans-NIH Workshop on non-mammalian models.

Dr. Heindel discussed the Small Business Innovation Research (SBIR) grant program. He explained that one focus of this funding is to create a small business partnership with academia that would translate existing research into marketable products. The process is separated into three phases beginning with the initial development phase. Phase II involves prototype development and Phase III consists of production and marketing. He then provided examples of SBIR grants that have been funded in the areas of alternative models (10), carcinogenicity and genotoxicity testing (9), and endocrine disruptor testing (7). Dr. Heindel gave examples of "success stories" from SBIR-funded research including the Stratagene Big Blue Mouse, MatTek's cultured corneal epithelium, Gentest's genetically engineered cells with metabolic activity, and Oxford Biomedical's antibodies for drug metabolizing enzymes.

Dr. Heindel concluded his presentation by discussing the future directions of the Extramural Division with respect to alternative models. He said that SBIR grants and contracts and RFAs for FY 2001 are currently in the planning stages, but that they will hopefully help to stimulate validation efforts. He listed several alternative models, which may surface in the future, and presented a process of validation for alternative models, beginning with grant-funded research for model development. He concluded by presenting a 12-step process for the development, validation, acceptance, and use of new test methods.

Discussion:

Dr. McClellan stated that he liked the 12-step process diagram and especially appreciated step 12 as usage, because that is ultimately most important. Looping from step 12 back to step 1, it becomes evident for the need to have all test requirements assembled in one place, thereby allowing the University investigator to access them. He suggested that a SBIR be considered that is broad in terms of the 3Rs, to address the two major sets of testing guidelines, EPA's Health Effects Guidelines and FDA's Redbook.

Dr. Green stated that he was pleased with Dr. Heindel's presentation and recommended that reproductive toxicology should be considered for future alternative models, especially with the availability of embryonic stem cells. Dr. Heindel stated that it would be a good idea to identify

this suggestion as a goal so that it might be considered for future funding. He suggested that a cooperative agreement might be an effective way to achieve progress on such an effort.

Dr. Hurt stated that the vision of ICCVAM and the Advisory Committee is to ultimately foster the development of new test methods to provide better predictions of toxicity. ICCVAM and the NTP Center have established an effective process for considering test methods that have already been developed and this approach has been a huge success; these methods are now entering the regulatory testing process. She proposed that it is time to begin fostering the development of the new methods and developing a strategy. For example, explore one or two new technologies, such as microarrays, proteonomics, or genomics, and begin with a workshop to investigate the steps to bring this technology into regulatory use. Next, funding should be provided to begin the research to fill those identified knowledge gaps and move forward. People aware of the capabilities of the technology and those educated in the means of regulatory assessment should be working together to attain these goals. Dr. Curren added that it is very important to focus on how the technology can be used to improve the predictions of a specific toxic endpoint; further, knowledge pertaining to how to utilize the information that comes from, for example, gene expression type assays, to improve hazard identification is necessary. He asked if it would be possible for Dr. Heindel to update the Committee with a more in-depth summary on an annual basis as to the alternative efforts being funded so that they could provide feedback from their perspective. Dr. Heindel responded that he would be pleased to provide such updates.

Dr. Curren stated that he agreed with Dr. Stitzel that the focus for chip technology should not be the final goal, but to focus on a toxic endpoint as a starting point. He reaffirmed Dr. Curren's suggestion of an annual summary of alternative method funding.

Dr. Curren stated that a critically important step in alternative development is to provide chemical supply and characterization support for validation and prevalidation studies. This approach would include coding, distribution, and characterization of test materials. A second broad need is for the acquisition and maintenance of a good human toxicity databank. It may be easier to break down the database into smaller pieces to allow for easier funding, but that such reference data are essential in validating methods that will predict human toxicity.

Dr. Denison stated that with respect to microarray technology, the ability to associate differential gene expression with toxicological endpoints is needed. He stated that people are generating massive amounts of data using microarray technology to explore gene expression; if this information is ever to be used for risk assessment or toxicological endpoint determination, then these applications should be considered now. Dr. Denison also stated that he agreed with Dr. Curren that high quality chemicals with careful analyses are essential for successful validation studies, particularly for such studies as endocrine disruptors.

Dr. Denison also added that he actually had one of the original grants from the RFA on alternative bioassays. It generated data that has now been used to obtain a new grant and an SBIR, both phase I and II. He stated that the targeted RFAs do work to develop new alternative methods.

Dr. Stitzel commented that with respect to Dr. Denison's point regarding the association of differential gene expression with toxicological endpoints, the real need is for some kind of quantitative risk to be involved rather than just hazard identification, in order to relate the hazard to dose response, including no effect and lowest effect dose levels. Dr. Stitzel then suggested that microarrays would be a good grant subject so that this work could be carried out. Dr. Stokes added that a strategic plan would be helpful to advance this approach. Dr. Stitzel recommended that an initial strategic plan should be developed that could be critiqued, modified, and then updated as progress is made.

Dr. Montgomery suggested that support for the development of mouse endothelial cell lines would be a good way to advance research in environmental toxicology. He also stated his support for funding the development of databases that combine data from more than one science, such as genomic data, histopathology, and gross pathology. Dr. Ramos added that this approach would be an enormous resource for the academic community. Dr. Heindel stated that Dr. Paules' group is attempting to develop such a database containing microarray data, toxicity endpoints, tissues, and other information.

Dr. Ramos stated that a major issue for the integration between microarray technology and classical toxicological endpoints would be involvement of the proper investigators. He added that there is a need for administrative guidance and suggested a directed workshop that presents actual problems that need to be addressed scientifically. He stated that the resulting action items could then direct the movement of the process. Further, a deficiency in the RFA program is the lack of understanding on behalf of the grant review committees as to the mission and the ultimate goals. A clear idea of research needs is necessary so that when the grant comes to the review committee, the members are familiar with the needs and the grant will not be lost. This premise is also beneficial to the review committee, as the information conveys the message of need. He also suggested that more effort be invested into the dissemination of information to directors and staff members, notifying them of voids in the database.

Dr. McClellan asked if the NIEHS Extramural Center had been recently briefed on the goals of NICEATM and ICCVAM. Dr. Stokes stated that they had not been briefed recently, but that he had taken part in a meeting with the 11 grantees for the alternative methods to inform them on the validation process and regulatory testing requirements. He stated that it is important to continue this for future RFAs.

Dr. Rowan commented that an option would be to write an RFA that outlined the critical disciplines that had to be involved in a proposal, such as risk assessors, molecular biologists, and pathologists. Dr. Ramos added that RFAs are generally good, but that there is a tendency to run into problems with the review committee.

Dr. Heindel stated that efforts have been made to establish guidelines for review committees, but that the review process is a totally separate process that functions on its own. Dr. Stitzel suggested devising a different method for reviewing alternative method grants should this process continue to be a problem.

Dr. Goldberg added that many of these committees are anti-alternative and anything related to alternatives would be denied. Dr. Heindel stated that this would not be the case if the proposal were set up as a targeted RFA for alternative models. The reviewers would then be from backgrounds involving alternative methods.

Dr. Bucher stated that he wanted to clarify that the NTP has the chemistry resources and capabilities of providing analyzed chemicals that could be sent out under code. He added that the proposal of a workshop to consider the steps between microarray data generation and risk assessment is similar to the 1995 workshop on transgenic animals. He stated that this workshop included people from academia, government, regulatory agencies, and the legislative branch. He said that everyone must be oriented in the same manner and that although this is not an easy task, it can be done.

X. Open Discussion: Future Directions

Dr. Stitzel opened the floor for discussion regarding the information presented over the past two days; she stated that this session was the opportunity for the committee to provide advice to ICCVAM regarding future directions for their work and test method development and validation. She stated that the gene microarray technology was an example of the unclear procession from the current research mode to the application of this information to quantitative risk assessment.

Dr. McClellan stated that a workshop would be a useful starting point in that area, with participation from individuals in the risk analysis community, molecular biology community, and individuals that are trained and understand pathobiology at the level of laboratory animals and humans. Such a workshop would be useful for exchanging ideas and could serve as the basis for a RFA. He also encouraged the staff to be thinking in terms of creating a strategic plan for the NTP Center beginning with the mission and vision. Such a plan should address strategy for future progress and the resources necessary in terms of dollars and staffing to include both government employees and contractors. He stated that, "the challenge is attaining the proper level of orientation since this is not a precise process; however, we need a means to recognize things that will change and developments that will occur."

Dr. Stitzel summarized that there is a need for two activities. First, a workshop is needed to develop a strategy for the gene chip technology and second is the need for the Center to develop a strategic plan. She stated that the most recent strategic plan focused on getting test methods through the evaluation process and gaining their acceptance, which has been successful. Presently, our next long-term strategy comes into question. She added that the Committee's discussions indicate that there are other activities that would be very useful and may involve significant resources, but the Committee would like to have a plan that they could comment on.

Dr. McClellan replied that the presentation this afternoon emphasizes that an important part of the Center's strategy appears to be linkage to the rest of NIEHS/NTP as well as the activities of other government agencies.

Dr. Stokes added that with respect to microarray technology, one of the questions posed to the FETAX expert panel is how microarray technology might be applied to improve the predictivity of FETAX. Another question to the panel relates to how knowledge of the *Xenopus* genome might be incorporated into the assay. He stated that ICCVAM has already begun asking the panel members to consider these future technologies and has included panel members with a wide range of expertise to cover these issues. These expert panel meetings and workshops will be developing a strategic plan for a specific test method, or in some cases, for a specific endpoint.

Dr. Theran stated that this is a new and exciting level of biology. He suggested that a strategy be developed to ensure good public interaction with respect to the status of method development and the proposed use of such methods, as misinformation tends to drive a wedge between scientists and animal advocacy groups. He added that part of the strategy should be the development of informative descriptions that can be understood by the public and provide good factual descriptions of where we are, where we are going, and what it will take to get there. Dr. Stitzel said that the other side of this approach is that industry fears there will be much emphasis on a gene that predicts something and the race to identify it. She stated that the same kind of clearly worded statements would be helpful on this side as well.

Dr. Hurt added that during its first two years, ICCVAM has been successful in attaining the first objective, which was to implement a process for bringing test methods into the regulatory arena. She then stated that the current focus needs to be fostering the development of new testing methodologies.

Dr. McClellan pointed out that there seems to be clarification in the step-wise approach of the process. He said that within the NTP and NIEHS, the joining of molecular technology and risk analysis should be a core program that goes forward. Furthermore, NIEHS needs to be the focus for joining new technologies to risk assessment needs.

Dr. Stitzel stated that there is an interest in international involvement and asked if there is anything that ICCVAM can do to refine how test methods are accepted internationally. Dr. Stokes replied that the two methods that have been thoroughly evaluated by ICCVAM will move through the OECD process in the near future. We are cautiously optimistic that the thorough ICCVAM evaluation process should expedite review and adoption by the 29 OECD member countries. He added that it would be helpful to harmonize the information that authorities need submitted to substantiate the validity of a method. For example, if some form of the ICCVAM submission guidelines could be adopted by OECD as well as ECVAM, then the same type of information would be provided for a test method, no matter where in the world it was developed or reviewed. He added that the information provided in the submission is critical for the evaluation process. He said that currently, the evaluation process is different in Europe than it is in the U.S. For example, in the EU there is no apparent opportunity for public comment or a public review process for proposed new methods, which are considered to be essential aspects of the ICCVAM review process. He stated that it might take time for these differences to be lessened and a harmonized process to evolve.

Dr. Goldberg asked if ECVAM has seen the submission guidelines so that they can compare it to its own guidelines. Dr. Stokes stated that Drs. Balls and Spielmann attended the October ICCVAM meeting and took part in the discussion on the revised the submission guidelines. He stated that he hoped that the OECD guidance document on this topic would incorporate the submission guidelines. Dr. Goldberg suggested that a follow-up letter be sent to ECVAM and ZEBET with the final submission guidelines.

Dr. Curren stated that with respect to harmonizing test guidelines, the criteria have never been established and that there has been no agreement as to the level of precision, etc. He said that until this has been established, harmonization couldn't be reached.

Dr. Stitzel stated that she wanted to stress the Committee's interest in developing a central repository of regulatory guidelines for test methods that would allow test method developers to know the task at hand. She stated that she was pleased that ICCVAM has been open to new technologies and that flexibility would be very important in the future.

Dr. Goldberg stated that he would like to see a report from the FDA and U.S. EPA regarding the change and utilization of the accepted methodologies that shows if the alternative methods are actually being incorporated. Dr. Stokes stated that one way to monitor change would be to review the USDA reports on animal use for species covered under the AWA. Column E listings would provide information on numbers of animals used in procedures involving unrelieved pain and distress. For instance, the FY99 USDA Report listed over 10,000 guinea pigs in Column E that were used for hypersensitivity testing.

Dr. Rowan said that he believed the USDA numbers are useless and provide little or no guidance. He added that the UK numbers are slightly more helpful. Dr. Stitzel replied that she was of the opinion that the USDA numbers are correct, but that the pain classification categories are not accurate. Dr. Rowan agreed that the information is probably more accurate now, but that the classifications are completely off base.

Dr. Rowan stated that it was clear, based on Dr. Heindel's presentation, that traditional investigator initiated grants have not worked well to develop new test methods. He added that RFAs are useful, but what is really needed is targeted research. NIEHS and NTP were directed to

establish new methods; this action requires a different process than RO1 grants and we need to start thinking about a new process to drive forward from this point. Test method standardization, pre-validation, and validation require different support than the traditional funding mechanisms for grants. A method that is somewhat developed needs to be standardized to ensure it is robust and transferable. He stated that this approach equates to contract work, not an RFA or a RO1 and that a different process is needed. Dr. Rowan stated that project grants seem to be more successful because they involve groups competing against each other. He said that RFAs would not be as successful because they fail to bring together the correct people; further, ICCVAM needs to focus on using the correct process and utilizing qualified individuals from across the country. Dr. Ramos stated that NIEHS seems to be developing strategies for consortia, which is a good way for identifying expertise and bringing individuals together to form a cohesive unit.

Dr. Stitzel called for any additional comments from the panel or any public comments. Dr. Hurt expressed her appreciation for the privilege of having worked with the NTP and the privilege of having been involved from the beginning of the establishment of the Committee and the NTP Center.

Dr. Stokes thanked the Committee for their thoughtful comments and suggestions. He added that the Committee's recommendations will be helpful to the Center in moving from a reactive to a more proactive approach, and that he appreciated the Committee's advice on this new direction.

Dr. Bucher expressed his gratitude to the committee on behalf of the NTP and NIEHS for their participation stating that their comments will certainly be considered and that he hoped that many of the proposed initiatives could be pursued. He stated that this meeting had been very helpful and thanked everyone for their participation.

The meeting was adjourned at 4:30 p.m.